Glycated nail proteins as a new biomarker in management of the South Kivu Congolese diabetics

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Abstract

Introduction: Diagnosis and monitoring of *diabetes mellitus* in sub-Saharan Africa, based on blood analyses, are hampered by infrastructural and cultural reasons. The first aim of this study was to evaluate the diagnostic accuracy of glycated nail proteins for diabetes mellitus. The second aim was to compare the course of short- and long-term glycemic biomarkers after 6 months of antidiabetic treatment. These objectives should support our hypothesis that glycated nail proteins could be used as an alternative glycemic biomarker.

Materials and methods: This case-control study consisted of 163 black diabetics and 67 non-diabetics of the South Kivu (Democratic Republic of Congo). Diagnostic accuracy of glycated nail proteins was evaluated using ROC curve analysis. At the start of the study, glycated nail protein concentrations were compared between diabetics and non-diabetics, using a nitro blue tetrazolium (NBT) colorimetric method. In a subgroup of 30 diabetics, concentrations of glycated nail proteins, fasting glucose (Accu-Chek® Aviva), serum fructosamine (NBT) and HbA1c (DCA-2000+®) were measured at start and after 6 months.

Results: ROC analysis yielded an AUC of 0.71 (95% confidence interval (CI): 0.65-0.76) and a cut-off point of 3.83 µmol/g nail. Concentration of glycated nail proteins was significantly higher ($P < 0.001$) in diabetics in comparison with non-diabetics. After 6 months of antidiabetic treatment, a significant drop in the fasting glucose concentration ($P = 0.017$) and concentration of glycated nail proteins ($P = 0.008$) was observed in contrast to serum fructosamine and HbA1c.

Conclusions: Measurement of glycated nail proteins could be used to diagnose and monitor *diabetes mellitus* in sub-Saharan Africa.

Key words: fasting glucose concentration; fructosamine; hemoglobin A1c protein; nails; sub-Saharan Africa; diabetes mellitus

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Introduction

Besides the high rates of communicable diseases, an increasing prevalence of *diabetes mellitus* is observed in sub-Saharan Africa (1). In comparison with the current 19.8 million diabetics in this region, this number is expected to double by 2035 (2). Screening and monitoring of *diabetes mellitus* is of utmost importance as it is associated with an important morbidity (heart disease, nephropathy, neuropathy, and retinopathy) and mortality, as well as with a negative influence on the local economies. In 2013, approximately 75% of all adult deaths due to *diabetes mellitus* in less-developed

regions, like sub-Saharan Africa, were in people under 60 years old (3).

Although hyperglycemia remains the biochemical hallmark of diabetes mellitus, several other glycemic markers (fructosamine, glycated albumin, hemoglobin A1c (HbA1c), 1,5-anhydroglucitol) have been proposed for the diagnosis and follow up of this disease, each with their own advantages and limitations (4). Although HbA1c is the best surrogate of the average blood glucose control in diabetics, this measurement is unavailable in most parts of Africa. Taking into consideration the lack of infrastructure and the aversion to blood sampling in some populations of rural Africa, the measurement of glycated proteins (fructosamine) in human finger nails could be excellently suited for low-income countries. Fructosamine (1-amino-1-deoxy-D-fructose) is the result of a non-enzymatic reaction of the carbonyl group of glucose with an amino group of circulating serum proteins. As the protein content of human nails is about 80%, the physiological fructosamine/protein ratio is in the order of 2-2.5 µmol/g nail protein. In human nail clippings, a pure protein matrix in absence of interfering substances is found, which is ideal for fructasmine testing.

The first aim of the study was to evaluate the diagnostic accuracy of measuring the concentration of glycated nail proteins for *diabetes mellitus* in a sub-Saharan African population. The second aim of the study was to compare the course of short-term (fasting glucose concentration and serum fructosamine concentration) and long-term (concentration of glycated nail proteins and HbA1c level) glycemic biomarkers after 6 months of antidiabetic treatment. This study should make it possible to evaluate the hypothesis if glycated nail proteins could be utilized for the diagnosis and monitoring of *diabetes mellitus* in sub-Saharan Africa.

Materials and methods

Subjects

This case-control study was performed between 1 June 2013 and 1 June 2014 in Bukavu, a city in the Democratic Republic of Congo (South Kivu province). African patients with *diabetes mellitus* (novel diagnosis or under antidiabetic treatment) and with an HbA1c of ≥ 48 mmol/mol (6.5%)), consulting the department of Internal Medicine of the Reference Provincial General Hospital of Bukavu, were included in the study. Diabetics already under treatment and with an HbA1c of < 48 mmol/mol (6.5%) were excluded from the study. According to the current World Health Organization (WHO) diagnostic criteria, an HbA1c value of 48 mmol/mol (6.5%) was used to identify new patients with diabetes mellitus (5). The control group consisted of 67 healthy, non-diabetic African students of the Faculty of Medicine of the Catholic University of Bukavu, who underwent a general clinical examination and a blood analysis, and did not take any medication. After signing the informed consent, 230 subjects were involved in the study: 163 diabetics (87 females, 76 males, median age: 59 years, range: 13-87 years) and a control group (26 females, 41 males, median age: 22 years, range: 20-77 years). The concentration of glycated nail proteins was compared between both groups. In a subgroup of 30 diabetic patients (14 females, 16 males, median age: 57 years, range: 14-87 years), the concentration of two short-term (fasting glucose concentration and fructosamine) and two long-term (glycated nail proteins and HbA1c) glycemic biomarkers was measured at start and after 6 months of antidiabetic treatment. The procedures were performed in accordance with the ethical standards set by the responsible human ethics committee (Ghent University hospital BUN: B670201215602) and the 1975 Helsinki Declaration.

Methods

Fasting blood samples were collected in the morning by trained nurses and processed for biochemical determinations. The concentration of capillary blood glucose was determined by an Accu-Chek® Aviva (Roche Diagnostics, Mannheim, Germany). HbA1c was assayed on K2-ethylenediaminetetraacetic acid (EDTA) blood specimens (Becton Dickinson, Nairobi, Kenya), using a DCA-2000+® analyzer (Siemens, Marburg, Germany). Fructosamine (1-amino-1-deoxy-D-fructose) was measured

in serum samples (Becton Dickinson, Nairobi, Kenya) and in nail fragments using a nitro blue tetrazolium (NBT) colorimetric method and a commercial fructosamine standard (Roche Diagnostics, Mannheim, Germany) (6).

Distal human finger nail fragments were collected using a classic nail clipper. The size of the nail fragments ranged from 0.3 to 3.0 mg *per* fragment. As previously demonstrated (4), treatment with detergent (soap) had no influence on the measured concentration of glycated nail proteins. After weighing, the nail clippings were transferred into a standard 10 mm pathway cuvette. One mL of fructosamine reagent (0.25 mmol/L NBT (Sigma, St. Louis, MO, USA) in a 0.1 mol/L sodium carbonate/bicarbonate buffer (pH: 10.3), containing 0.1% Triton X-100 (Fluka, St. Louis, MO, USA)), was added to the nail clippings. Following incubation (37 °C, 60 min), photometric readings occurred at 530 nm in a UV-1800 photometer (Shimadzu, Kyoto, Japan). The spontaneous rate of hydrolysis of the NBT dye is very low and below the detection limit within the observation period (60 min). A commercial fructosamine standard (Roche, Mannheim, Germany) was used for standardizing the assay. For internal quality control, commercial fructosamine standards (Roche Diagnostics, Mannheim, Germany) have been assigned. Results were expressed as µmol of fructosamine *per* g of nail. The within-run CV of the assay was 11%.

The blood analyses and the measurement of glycated nail fragments were performed once in all subjects at the start of the study. However in a subgroup of 30 diabetic patients, this procedure was repeated after 6 months of antidiabetic treatment.

Statistical analysis

All values are expressed as mean ± standard deviation (SD) (when data were normally distributed) or median ± IQR (when data were not normally distributed). Receiver operating characteristics curve (ROC) analysis was used for calculation of cut-off values. The normality of the data distribution was evaluated by the Kolmogorov-Smirnov test. For comparison of continuous variables, non-parametric Mann-Whitney U test was used. For the analysis of the paired data in the subgroup of 30 diabetics, paired Student's t-test was used, as the data were normally distributed. A P-value < 0.05 was considered to be statistically significant. Statistical analyses were performed using MedCalc® (MedCalc Version 15.5, Mariakerke, Belgium).

Results

ROC analysis yielded an AUC of 0.71 [95% confidence interval (CI): 0.65-0.76] and a cut-off point of 3.83 µmol/g nail (corresponding with a specificity of 68.3% and a sensitivity of 67.2%). At the start of the study period, the concentrations of fasting blood glucose, serum fructosamine, HbA1c and glycated nail proteins were significantly higher (P < 0.001) in the group of diabetic patients in comparison with the non-diabetics (Table 1).

In a subgroup of 30 diabetic patients, the course of the concentration of two short-term (fasting glucose concentration and serum fructosamine concentration) and two long-term (glycated nail protein concentration and HbA1c value) glycemic biomarkers was documented at start of the study and after 6 months of antidiabetic treatment (Table 2).

Table 1. Comparison of biochemical parameters between patients with *diabetes mellitus* and non-diabetics.

Biochemical parameter	Diabetes (N = 163)	Non-diabetics (N = 67)	P-value
Fasting blood glucose (mmol/L)	9.7 (7.5-14.8)	4.7 (3.7-5.2)	< 0.001
Serum fructosamine (µmol/L)	422 (333-540)	280 (237-350)	< 0.001
Hemoglobin A1c (mmol/mol)	73 (59-98)	33 (31-36)	< 0.001
Glycated nail protein (µmol/g nail)	5.30 (2.80-9.12)	2.90 (1.42-4.97)	< 0.001

Table 2. Comparison of biochemical parameters between patients with *diabetes mellitus* initially (month = 0) and after 6 months of antidiabetic treatment.

Biochemical parameter	Diabetics, month 0 (N = 30)	Diabetics, month 6 (N = 30)	P-value
Fasting blood glucose (mmol/L)	8.2 ± 4.2	7.2 ± 2.0	0.017
Serum fructosamine (µmol/L)	443 ± 113	367 ± 218	0.113
Hemoglobin A1c (mmol/mol)	71 ± 21	67 ± 23	0.306
Glycated nail protein (µmol/g nail)	6.5 ± 4.9	3.8 ± 2.5	0.008

A significant drop in the fasting blood glucose concentration (P = 0.017) and glycated nail protein concentration (P = 0.008) was observed in contrast to the non-significant changes in the serum fructosamine concentration and the HbA1c level.

Discussion

In an African district hospital, we have demonstrated for the first time the value of measuring the concentration of glycated nail proteins for the diagnosis of *diabetes mellitus* in a South Kivu Congolese population group. Although HbA1c has been incorporated into the new guidelines to diagnose *diabetes mellitus* (7), the use of this biomarker in Africa is not only hampered by infrastructural and financial problems. The presence of haemoglobinopathies (e.g. thalassaemias and sickle cell anemia) could influence the interpretation of the measured HbA1c levels and population-specific optimum cut-off points might be necessary for different ethnic groups and populations (8-11). In addition, the use of the proposed alternative glycemic biomarker could be very useful in developing countries as it has a less critical preanalytical phase and can be performed with low reagent costs.

In addition, the present study shows that the measurement of glycated nail proteins could be used to monitor *diabetes mellitus* over a long time period in black subjects of sub-Saharan Africa. Finger nails grow slowly at approximately 2-3 mm/month with a complete replacement performed between 6 and 9 months. This period allows a long exposure of the nail to the glycemic environment. As demonstrated in previous experiments (12), glycation of nail proteins takes place in the deep layer of finger nails. Using a hand piece containing a latch-type-bur, a meticulous cutting of the nail plate into superficial and deep layers was performed. Measuring the concentration of fructosamine in the superficial and deep nail layers, significantly lower glycated nail protein concentrations were found in the superficial nail layer in comparison with the deep layer. In contrast to the superficial nail layer, the deep layer is in close contact with the blood vessels and the interstitial fluid.

Discordances between HbA1c and other glycemic biomarkers have already been reported (13). The lack of a significant correlation between the concentration of glycated nail proteins and the serum fructosamine concentration and the HbA1c values could be explained by several reasons: a different exposure time to extracellular glucose (> 6 months for glycated nail proteins *versus* 10-14 days for serum fructosamine and 120 days for HbA1c), the unique valine linked glycation of HbA1c and the variable glucose diffusion rate from the vascular system to the nail bed (12). As demonstrated previously (6), the positive correlation between the concentrations of glycated nail and eye lens proteins suggests that the presented biomarker has also the potential to predict diabetic glycation-associated target organ damage.

A first limitation of this study was the monocentric character. A second limitation was that the fasting blood glucose concentration was determined on capillary blood and that we did not perform an oral glucose tolerance test to diagnose *diabetes*

mellitus. Finally, the course of the different short-term (fasting glucose concentration and serum fructosamine concentration) and long-term (glycated nail protein concentration, HbA1c level) glycemic biomarkers was only followed in a small subgroup of diabetics. Large-scale prospective clinical trials are necessary to confirm our results.

To conclude, measurement of glycated nail proteins could be used to diagnose and to monitor *diabetes mellitus* in black subjects of sub-Saharan Africa.

Potential conflict of interest

None declared.

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